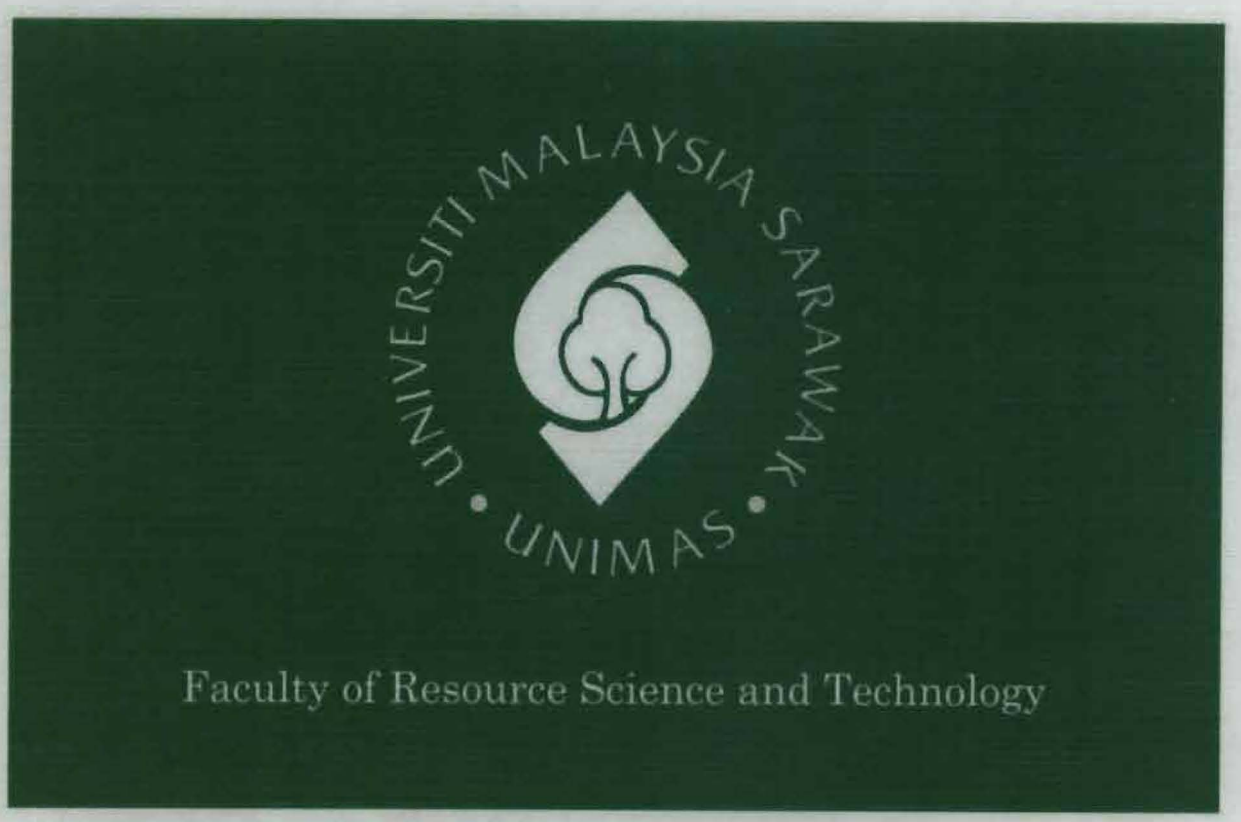

PHYTOCHEMICAL STUDIES AND PHARMACOPHORE ANALYSIS OF *SONNERATIA CASEOLARIS* FRUITS

Liyana Zafirah Binti Othman

Bachelor of Science with Honours
(Resource Chemistry)
2016

Phytochemical Studies and Pharmacophore Analysis of *Sonneratia caseolaris*'s Fruits

Liyana Zafirah Binti Othman

(41916)

This thesis is submitted in fulfillment of the requirement for the degree of

Bachelor of Science with Honours

(Resource Chemistry)

Supervisor: Dr. Mohd Razip Bin Asaruddin

Faculty of Resource Science and Technology

University Malaysia Sarawak

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LIST OF ABBREVIATIONS

DCM	Dichloromethane
$CHCl_3$	Chloroform
CD_3OD	Deuterated methanol
EtOAc	Ethyl acetate
TLC	Thin Layer Chromatography
CC	Column Chromatography
R_f	Retention factor
FT-IR	Fourier Transform Infrared Spectrophotometry
NMR	Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
^{13}C-NMR	Carbon Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass Spectrometry
GC-FID	Gas Chromatography-Flame Ionization Detector
UV-Vis	Ultraviolet Spectrophotometer
µL	microliter
LC_{50}	Lethality concentration
Ppm	Part per million
mL	Milliliter
CADD	Computer-Aided Drug Design
SBDD	Structure-Based Drug Design
LBDD	Ligand-Based Drug Design
MeOH	Methanol
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HI	Hydrophobic Interaction
AR	Aromatic Interaction
NIA	Negative Ionization Area

3-D	Three dimensional
S. caseolaris	*Sonneratia caseolaris*
E. coli	*Escherichia coli*
S. aureus	*Staphyloccocus aureus*

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Phytochemical Studies and Pharmacophore Analysis of *Sonneratia caseolaris*'s Fruits

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ABSTRACT

The aim for this study was to isolate the bioactive molecules, determine the biological activities and find the potential lead in *Sonneratia caseolaris* as antimicrobial drugs. Phytochemical examination on the methanol extract of the fruits gave about 37.37 g of crude and 4.82 % percentage yield which led to the isolation and characterization of compound 1. This structure compound were elucidated and characterized by spectroscopic analysis by using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy, and Fourier Transform Infrared Spectroscopy (FTIR). In ligand-based pharmacophore modelling, the interaction of compound 1 with generated pharmacophore model shows the presence of hydrophobic (HY) and hydrogen bond acceptor (HBA) pharmacophore features with fit value of 35.4800. Biological evaluations were carried out on the methanol and hexane crude extracts. The results stated that the methanol crude extracts has higher LC_{50} value that was 2344.0 ppm. This higher value indicated that compound has no toxicity towards *Artemia salina*. In disc diffusion for antimicrobial test, it was found that *Sonneratia caseolaris* crude showed weak inhibition zone (7.33 mm and 7.00 mm) towards *Escherichia coli* and mild inhibition towards *Staphylococcus aureus* for both methanol and hexane crude.

Keywords: *Sonneratia caseolaris,* antimicrobial, pharmacophore modelling, toxicity, biological evaluations

ABSTRAK

Tujuan kajian ini adalah untuk mengasingkan molekul bioaktif, menentukan aktiviti biologi dan mencari potensi dalam Sonneratia caseolaris sebagai ubat anti-mikrob. Kajian fitokimia atas ekstrak metanol daripada buah-buahan memberikan lebih kurang 37.37 g hasil peratusan mentah dan 4.82 % yang membawa kepada pengasingan dan pencirian sebatian 1. Sebatian struktur telah dijelaskan dan ciri-ciri analisis spektroskopi dengan menggunakan ^{1}H dan ^{13}C Nuclear Magnetic Resonance (NMR) Spektroskopi dan Fourier Transform Infrared Spektroskopi (FTIR). Berdasarkan ligan pemodelan pharmacophore, interaksi sebatian 1 dengan model pharmacophore dijana menunjukkan kehadiran hidrofobik (HY) dan ikatan hidrogen penerima (HBA) ciri-ciri pharmacophore dengan nilai patut 35.4800. Penilaian biologi telah dijalankan ke atas metanol dan ekstrak heksana mentah. Keputusan menyatakan bahawa ekstrak metanol mentah mempunyai nilai LC_{50} yang paling tinggi iaitu sebanyak 2344.0 ppm. Nilai ini lebih tinggi menunjukkan bahawa sebatian tidak toksik terhadap Artemia salina. Dalam penyebaran cakera untuk ujian antimikrob, didapati bahawa Sonneratia caseolaris mentah menunjukkan zon perencatan rendah (7.33 mm dan 7.00 mm) ke arah Escherichia coli dan perencatan sederhana ke arah Staphylococcus aureus untuk kedua-dua metanol dan heksana mentah.

Kata kunci:Sonneratia caseolaris, antimicrobial , pemodelan pharmacophore, toksik , penilaian biologi

1.0 INTRODUCTION

1.1 General introduction

Computer-aided Drug Design (CADD) is a computational methods and resources that are used to facilitate the design and discovery of new therapeutic solutions (Song *et al.*, 2009). It shows that search for new potent antimicrobial drug is essential to provide a safer treatment for the patients. A pharmacophore can be derived into two methods which are ligand-based and structure-based (Wobler *et al.*, 2008). However, it shows that there have been only a few studies on *Sonneratia caseolaris* regarding the pharmacological activities and discovery of new drug by using CADD. Therefore, this is an opportunity to develop and search for new potent antimicrobial drug from the fruit of *Sonneratia caseolaris* plant that could be commercialize.

Sonneratia caseolaris is one of typical mangrove tree species known as "kirala" that grows well in brackish water bodies in Sri Lanka (Abeywickrama & Jayasooriya, 2010) (Figure 1.1). In Malaysia, the fruit is known as "pedada" or "berembang". *Sonneratia caseolaris* belongs to the family Sonneratiaceae (Nazli & Hashim, 2010). Howlader *et al.*, (2012) had states that *Sonneratia caseolaris* species is widespread and can be found easily in Asia. Based on previous research, *Sonneratia caseolaris* old fruit walls are used for helmintic infections, half-ripe fruit for coughs, while the pounded leaves for hematuria and small pox (Rahmatullah *et al.*, 2012). In addition, studies state that the calyces of *Sonneratia caseolaris* exhibited the strong antioxidant activity then followed by stamens (Bunyapraphatsara *et al.*, 2003). As for toxicity test, it shows that the leaf of *Sonneratia caseolaris* methanolic was not toxic to *Artemia salina* (Melki *et al.*, 2011).

Previous studies states that steroids, flavonoids, triterpenoids and benzene carboxylic derivatives have been isolated and identified from the stems and twigs of *Sonneratia caseolaris* (Tian *et al.*, 2009). It shows that this plant contains high potential secondary metabolites that have medicinal value. The value of these secondary metabolites is increasing due to the constant discoveries of their potential role in drug development (Remani *et al.*, 2012). Figure 1.1 shows the plant that was discovered in this study.

Figure 1.1 *Sonneratia caseolaris* plants

1.2 Objectives

This study will cover the following objectives:

1. To extract and isolate the chemical compounds from fruits of *S. caseolaris* by using chromatography techniques.
2. To characterize and identify the chemical constituents by using Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance Spectroscopy (NMR).
3. To generate pharmacophore model by using commercial drugs.
4. To determine fit value of phytochemical constituents originate in *S. caseolaris* with commercial drugs by using the ligand-based pharmacophore modeling approach (LigandScout 3.1).
5. To determine the antimicrobial and cytotoxicity (*Artemia salina*) value of *S. caseolaris*'s fruit.

2.0 LITERATURE REVIEW

2.1 Computer-aided drug design (CADD)

Song *et al.*, (2009) states that Computer-aided drug design (CADD) is a specialized discipline that uses computational knowledge based methods to aid and facilitate the new drug discovery solutions. According to Kalyaanamoorthy & Chen, (2011) as for searching high affinity of flies repellent, a powerful molecular modelling technique has been useful in designing lead targets. To design good drugs, biological drug interactions should be considered such as using CADD to design a new drug of molecules in order to treat certain diseases in the human (McCarthy, 1999). The application of CADD was supported by using pharmacophore modelling. Chemical features based 3D pharmacophore models was built using computer software LigandScout 3.1 and used to evaluate and produced the geometrical structures of training set in their active forms (Figure 2.1). An established compound from Protein Data Bank (PDB) was used as training set. All structure of test set was generated and optimized using computer ChemDraw Ultra 12.0 software. Pharmacopore model were generated using a series of selected training set and then were mapped with test set. Figure 2.2 illustrated the work flow of pharmacophore modelling that will be used in this study.

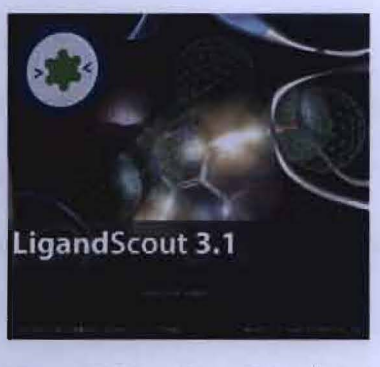


Figure 2.1 LigandScout 3.1 software

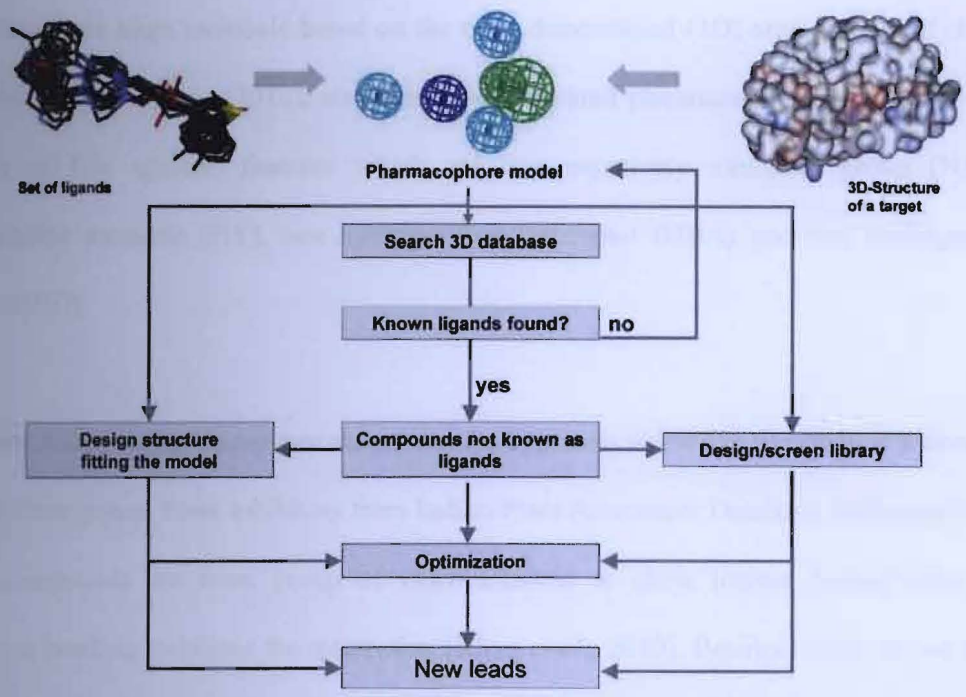


Figure 2.2 The workflow of pharmacophore modelling (Langer & Wolber, 2004)

2.1.1 Pharmacophore modelling

The pharmacophore can be derived both in a structure-based methods which between a ligand and its binding site, or in a ligand-based methods, which flexibly overlaying a set of active molecules and determining those conformations that are able to be overlapped in such a way that a maximum number of important chemical features geometrically overlap (Wobler *et al.*, 2008). Pharmacophore modeling approach is the technique rigidly models the interaction between a ligand and its binding site in a specific binding situation. The result is a three-dimensional (3D) spatial arrangement of chemical features. These feature maps, called 3D pharmacophores, can be used to search for similarities between binding situations or even for similarities between molecules.

Pharmacophore align molecule based on the three dimensional (3D) arrangement of chemical features. Noureen *et al.,* (2010), states that the generated pharmacophore hypothesis usually consist of five specific features which are one negatively ionizable group (NI), two hydrophobic aromatic (HY), one hydrogen bond acceptor (HBA) and one hydrogen bond donor (HBD).

Previous study on pharmacophore shows that the approach utilized in the study is successful in finding three potent Ftase inhibitors from Indian Plant Anticancer Database. Individually those three compounds are from group of vinca alkaloid & show lowest docked energy and hydrogen bonding stabilizes the interaction (Khan *et al.*, 2010). Besides, study shows that the discovery of ARRR.5 pharmacophore model was created by using indolizine derivatives acting as 15-LOX inhibitors. The hypothesis ARRR.5 might be considered as the best pharmacophore model because it displayed a good regression coefficient value of 0.9451, Pearson R 0.7439 and survival score 3.094 (Sharma & Kumar, 2015).

2.1.2 Structure-based drug design (SBDD)

Structure-based drug design (SBDD) is the one with computational method shows that it assists to enhance progress in discovery and refinement of therapeutic agents (Marrone *et al.,* 1997). According to Kuntz, (1992) structure-based drug design refers to the complex process of using the information contained in the three-dimensional, 3D structure of a macromolecular target and related ligand-target complexes to design novel drugs for important human diseases. Structural information about a given macromolecular target will leads to a better

understanding of its specific function and enables the design of small molecule ligands that can bind to the target.

Pharmacophore modelling is one of the SBDD applications where it used training set (protein) from literature and database search (RCSB Protein Data Bank) to generate a pharmacophore model. This pharmacophore modelling was carried out using LigandScout 3.1 software.

2.1.3 Ligand-based drug design (LBDD)

Ligand-based drug design is a method used in the absence of the receptor 3D information and it relies on knowledge of binding molecules to the biological target of interest. 3D quantitative structure activity relationships (3D QSAR) and pharmacophore modeling are the most important tools used in ligand-based drug design (Aparoy *et al.*, 2012).

2.2 *Sonneratia caseolaris*

S. caseolaris is one of typical mangrove tree species known as "kirala" that grows well in brackish water bodies in Sri Lanka (Abeywickrama & Jayasooriya, 2010). In Malaysia, the fruit is known as "pedada", "berembang", crabapple mangrove or mangrove apple. *S. caseolaris* belongs to the family Sonneratiaceae (Nazli & Hashim, 2010). *S. caseolaris* species is widespread and can be found in Bangladesh, Brunei Darussalam, Cambodia, China, India, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Viet Nam, Northeast Australia, Papua New Guinea, Solomon Islands, Vanuatu, New Caledonia, and Maldives (Md. S I. Howlader *et al.*, 2012). Table 2.1 shows the taxonomy of the *S. caseolaris* plants.

Table 2.1 Taxonomy of *Sonneratia caseolaris*

Kingdom	**Plantae (Plants)**
Division	Tracheophyta
Class	Magnoliopsida
Series	Myrtales
Family	Sonneratiaceae
Genus	Sonneratia
Species	*Sonneratia caseolaris*

Figure 2.3 The *S. caseolaris* fruit and leaves

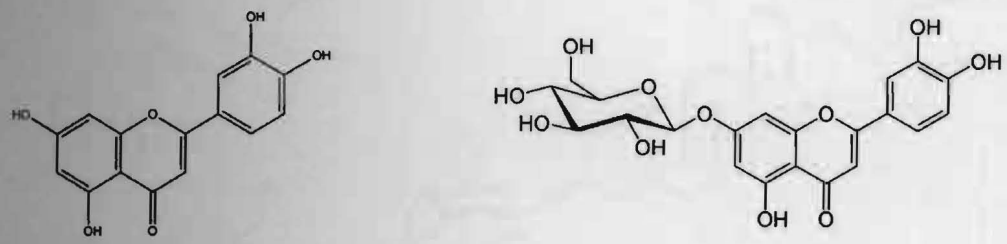
Ahmed *et al.*, (2010) has states that the mangrove tree has oblong or obovate-elliptic coriaceous leaves and large red flowers. The tree also can grow up to 15-20 meters high. This typical non-viviporous mangrove species have their own benefits such as the fruit is edible, sap is used as a skin cosmetic, and leaves are used for feeding goats (Avenido & Serrano, 2012) (Figure 2.3). The ripened fruits of *S. caseolaris* have an appealing flavor and taste that can be used to prepare a delicious fruit drink. The fruit also contains a large number of small

seeds embedded in the fleshy part which are bitter if eaten (Abeywickrama & Jayasooriya, 2010). The tree flowers are edible as vegetable with *nampriks* (spicy dish). "Pedada" is a sour tasting young berry fruits that are edible (Wetwitayaklung *et al.*, 2013). In addition, the mature fruits really have a cheese like taste and can be eaten raw or cooked. Its stem and branch are used for firewood, building boats, posts of bridges and houses. Other than that, it can absorb, accumulate, distribute and circulate that heavy metal such as Cu, Pb, Zn, Cr and Ni in mangrove community (Wetwitayaklung *et al.*, 2013).

2.3 Phytochemical constituents in *Sonneratia caseolaris* fruits

2.3.1 Flavonoids

Flavonoids is one of secondary metabolites group which responsible for antioxidant activity. Based on previous studies, two flavonoids such as luteolin (Figure 2.4a) and luteolin-7-*O*-β-glucoside (cynaroside) (Figure 2.4b) have been isolated from *Sonneratia caseolaris* leaves (Wetwitayaklung *et al.*, 2013).



(a) **(b)**

Figure 2.4 Example of flavonoid structures

2.3.2 Sterols

Sterols are also known as steroid alcohols which occur naturally in plants. Based on previous study, sterols compound have been identified from the stem and twigs of *S. caseolaris*. The sterols that have been isolated are 6′-*O*-Acetyl-β-daucosterol, β-Sitosterol (Figure 2.5a), Daucosterol (Figure 2.5c), β-Sitosterol palmitate (Figure 2.5d), Stigmast-5-en-3β-*O*-(6-*O*-hexadecanoyl-β-Dglucopyranoside, and Cholest-5-en-3β,7α-diol (Tian *et al.*, 2009). After that, Tiwari *et al.*, (2010) states that through extraction and isolation, bioactive compound from the methanol extracts of pedada fruit such as β-sitosterol-β-D-glucopyranoside were identified.

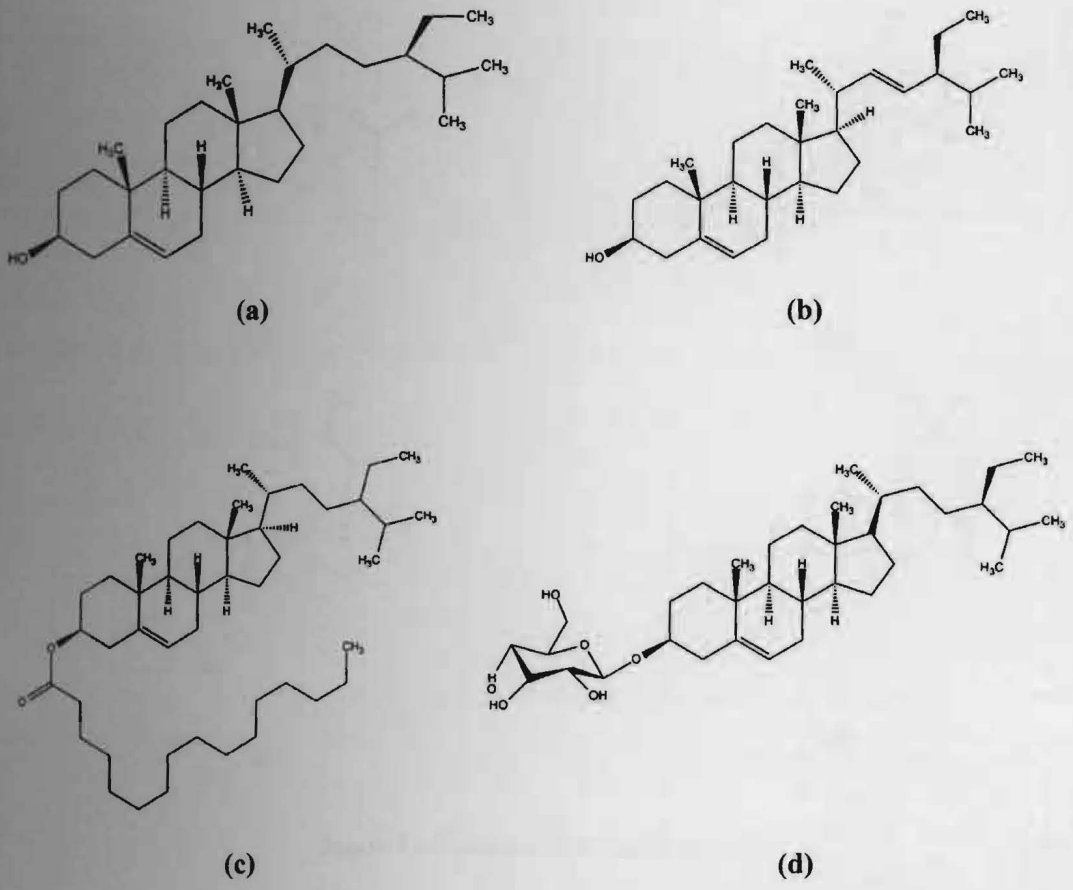


Figure 2.5 Example of sterol structures

2.3.3 Triterpenoids

Triterpenoids are one of the secondary metabolites that represent the largest group of phytochemicals and research shown that it can be found in various plants including fruits and medicinal herbs (Bishayee *et al.*, 2011). Previous study shows that maslinic acid, betulin, lupeol and oleanolic can be found in *S. caseolaris* plants (Figure 2.6). Besides, oleanolic acid is well known to possess anti-inflammatory, antihyperlipidemic and hepatoprotective activities and sold as oral drug for human liver disorder in China (Liu, 1995) (Figure 2.6d).